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14. ABSTRACT This report covers the fourth year of this project. At the conclusion of the original period of performance a no cost extension (NCE) was requested to allow samples to be analyzed by our industry collaborator, Metabolon, Inc. This request was delayed while issues raised by the Office of Research Protection Human Research Protection Office (HRPO) were addressed, and a protocol specific to this study was submitted to, and approved by the Johns Hopkins School of Medicine IRB. With the IRB approval, the issues raised by HRPO were resolved and the study was approved to continue. The NCE was granted, extending the period of performance to October 29, 2017, and authorizing use of previously unspent funds. The samples needed for analysis were identified from an IRB-approved bank of existing samples from men on active surveillance and the samples have been aliquotted and shipped to Metabolon, Inc. for analysis. The study is now on track to complete laboratory analyses in April 2017, biostatistical and bioinformatics analyses completed by the end of June 2017, and a manuscript submitted by the end of the period of performance in October 2017.					
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INTRODUCTION

This is the 4th Annual Report for this project. It provides a summary of activities during the period following the end of the original period of performance of the grant, including the request for a No Cost Extension that was described in the third Annual Report (dated May 29, 2015). The events leading to the request for NCE will be briefly summarized, followed by a description of the resolution, and subsequent progress.

BODY

NCE, HRPO and IRB issues

On April 24, 2014, Karen Eaton, from USAMRMC ORP HRPO, emailed Dr. Trock requesting clarification of IRB status for the project, because the project proposed to use samples from other, IRB-approved protocols led by other Principal Investigators (but on which Dr. Trock was an investigator). Dr. Trock ultimately sent Ms. Eaton a detailed response with requested documents on May 19, 2015 (attached as **Appendix 2**). Based on the information that Dr. Trock sent to Ms. Eaton she determined that Dr. Trock needed to submit a separate application to the Johns Hopkins School of Medicine (JHM) IRB. Because of this determination Dr. Trock was requested to halt study-related activities until the project with Dr. Trock as PI received IRB approval. Dr. Trock halted the study and submitted a protocol to the JHM IRB (attached as **Appendix 3**).

During this time, Dr. Trock submitted the 3rd Annual Report, describing the need for a NCE. The primary reason was that delays in obtaining the required samples (described in the 1st, 2nd, and 3rd Annual Reports) resulted in the project period of performance ending before samples could be submitted to the industry collaborator, Metabolon, Inc., to perform the metabolomics analyses that were central to the project. Funds that were allocated in the budget for the metabolomics analyses had not been spent and were returned to the DOD. The NCE request was for those funds to be made available for the metabolomics analyses, once the IRB issues were resolved and samples were available for the analyses. The NCE request was submitted to Joshua McKean on August 27, 2014. However, the NCE could not be approved until the IRB issues had been resolved to HRPO's satisfaction.

The protocol with Dr. Trock as the PI was approved by the JHM IRB on July 12, 2016 (approval letter attached as **Appendix 4**). HRPO issued approval of the protocol through an email from Nancy E. Englar, MHL, BSN, RN, CIP on July 27, 2016. The NCE was approved by USAMRAA on August 24, 2016 as indicated in an email from Michelle L. Cromwell on that date. The original period of performance was extended to October 29, 2017. A copy of the modified award is attached as **Appendix 5** (only pages 1-2 of this 18 page document are attached).

Resumption of Study

Although described in prior reports, the study aims are repeated here for continuity:

Aim 1. Evaluate whether a metabolomics profile for aggressive prostate cancer developed in tumor tissue by Metabolomics scientists can distinguish Gleason 6 vs. Gleason 7 tumors when measured in urine and serum from prostatectomy patients (n=50 patients from each group). The metabolomics analysis will not be confined to the metabolites in the existing profile, so there will be the potential for additional discovery analyses if the signature does not distinguish Gleason 6 vs. 7 cases when measured in urine or serum.

Aim 2. Determine whether the best performing metabolomic profile developed in Aim 1, when measured in baseline urine or serum samples (whichever performed best) from active surveillance men, can distinguish those who do vs. do not experience upgrading from Gleason 6 to Gleason 7 during follow-up (n=100 patients from each group).

At the time the study was halted, samples for Aim 1 had already been sent to Metabolon, Inc. (without any HIPAA-defined PHI). These samples comprised 80 patients with matched serum and urine, 21 patients with serum only, and 6 patients with urine only (total 187 samples). A series of discussions ensued with scientists at Metabolon, Inc. about the most rigorous approach to analyzing the samples. The issues were as follows. The samples came from 2 different cohorts: a study conducted by Dr. Partin that collected both serum and urine, and a study conducted previously by Dr. Trock that collected only serum (these studies described in 3rd Annual Report).

The Metabolon collaborators felt that potential differences in the Partin and Trock cohorts, and lack of both serum and urine for all patients could introduce batch effects and unwanted variability. This would be exacerbated by the fact that Aim 2 patients came from a 3rd cohort. A decision was made to not analyze the Aim 1 patients that were stored in freezers at Metabolon, and to perform discovery using both serum and urine from the Aim 2 patients. This would provide a stronger discovery platform and keep discovery in the clinical context that was the ultimate goal of the project, i.e. clinical decision-making in active surveillance. The Aim 1 samples could be stored until it was determined whether they could be useful for further or analyses, or ultimately returned to Dr. Trock.

Samples were identified from the active surveillance IRB-approved database managed by Dr. H. Ballentine Carter (IRB protocol #NA00045103 – described in 3rd Annual Report), and serum and urine from 100 patients in each of the 2 groups were aliquotted and shipped to Metabolon, Inc. in 2 batches on Feb 22 and Feb 27, 2017. The samples are now undergoing analysis at Metabolon.

REPORTABLE OUTCOMES

None

CONCLUSIONS

Issues raised by HRPO and have been resolved, and a separate protocol for this study with Dr. Trock as PI has been approved by the JHM IRB and HRPO. A NCE has been approved and the unspent funds authorized to pay for analyses (the original purpose of the funds), and all requisite samples have been shipped to Metabolon, Inc. for analysis. Metabolomic analyses are expected to be completed in April, and bioinformatics analyses conducted both by Dr. Trock and by Metabolon scientists will be completed by the end of June. This should allow submission of an initial manuscript by the end of the period of performance in October 2017.

1. APPENDICES

1. List of abbreviations and acronyms
2. Response to Karen Eaton 4/24/14 HRPO review
3. Application to JHM IRB, protocol #IRB00096293
4. IRB approval of protocol #IRB00096293
5. No Cost Extension approval (Amendment/Modification No. P00002), Aug 24, 2016
6. Abstracts, publications and manuscripts in preparation
7. Personnel receiving pay from this negotiated effort

LIST OF ABBREVIATIONS AND ACRONYMS

HRPO	Human Research Protection Office
JHM IRB	Johns Hopkins School of Medicine Institutional Review Board
NCE	No cost extension
PCBN	Prostate Cancer Biorepository Network
PHI	Protected Health Information

Response to HRPO review of Proposal PC100802 (Award W81XWH-11-1-0451)
Raised by Karen Eaton in email dated April 24, 2014

Ms. Eaton raised a number of questions regarding the IRB-approved protocols under which samples would be collected for PC100802. For completeness, her questions will be reproduced here followed by our responses. However, as a preface we will describe changes to the study that required us to change some of the sample sources, and thus, IRB protocols governing those sample collections.

The original Aim 1 of the study involved prospective collection of frozen prostatectomy (RP) tissue, and collection of paired serum and urine samples. The RP tissues were to come from IRB protocol #NA00048544 (original protocol application to the IRB “The Johns Hopkins Urological Specimen Repository and Database” (Angelo DeMarzo, PI), and the paired serum and urine samples were to come from IRB application #00-06-14-01/protocol #NA00047205 “Development and Evaluation of a Tumor Marker for Prostate Cancer (Alan Partin, PI). However, due to unanticipated difficulties in identifying samples that met the stringent study requirements and the unfortunate illness of the Research Nurse, Patricia Kolmer, there was substantial delay in identifying samples. These issues were described in the Year 2 Progress Report for award W81XWH-11-1-0451. During this period of delay, our industry collaborator who will perform the actual metabolomics analyses on this project (Metabolon, Inc.) identified a metabolomic profile associated with aggressive prostate cancer, derived from prostate tissues from 2 distinct patient cohorts with a larger sample size than we had originally proposed in our study. Thus, we changed Aim 1 from our original intent of discovery to identify a metabolomic signature in tissue that distinguished low vs. high Gleason grade, we now propose to validate - in urine or serum – whether the metabolomic signature identified by Metabolon could distinguish Gleason grade 3 from Grade 4.

Because this revised aim no longer required tissue we no longer needed specimens from Dr. De Marzo’s repository, and would use only existing serum and urine that was prospectively collected by Dr. Partin’s team under his protocol NA00047205.

Karen Eaton’s questions (in italics) and responses

Please address how the protocol entitled “Development and Evaluation of a Tumor Marker for Prostate Cancer” (PI: Partin) fits into the current DoD funded proposal. I have multiple IRB approval memos and a consent form on file for that study, but am unable to figure out where/how it fits.

Dr. Partin’s protocol prospectively collects paired blood and urine samples for men diagnosed with prostate cancer for the purpose of unspecified biomarker research, i.e. the samples are

collected to be made available for future studies as needed. Thus, although the samples are prospectively collected, our DoD proposal will only use existing samples that had already been collected at the time we changed our aim from discovery in frozen tissue to validation in serum and urine. However, we could only identify 86 patients (out of the 100 needed for aim 1) meeting the requirements of our study in Dr. Partin's existing sample. Therefore, we decided to obtain the remaining samples from existing samples collected under a separate ongoing protocol of Dr. Trock's, WIRB protocol #20011642 "Molecular Epidemiology of Prostate Cancer." This protocol also collects samples to be used for future unspecified biomarker studies. The additional samples needed were identified from among the existing samples of WIRB #1745, and now all samples for Aim 1 have been sent to Metabolon.

Aim 2 of PC100802 has not changed and will use existing samples that were prospectively collected from men in the John Hopkins Active Surveillance program under IRB protocol #XXXX "Active Surveillance for Prostate Cancer" (PI: H. Ballentine Carter). Therefore all samples for the aims of PC100802 are existing samples collected under other IRB-approved protocols that permit collection and storage of samples for future studies to predict outcomes in men with prostate cancer.

Please confirm if the protocols submitted in your 10 April 2014 email (De Marzo, Trock, and if related, Partin) are receiving DoD funds from the CDMRP funded proposal PC100802 to complete their research activities. If possible, address what protocol activities are being funded from this proposal.

None of the 3 protocols named in response to question (a) above that provide specimens to proposal PC100802 have or will receive funds from this proposal, as all specimens were collected using funds specific to those protocols.

Please note that if each of the 2 (possibly 3P collection protocols are receiving DoD funds, then each will require submission of full protocol packages (currently approved protocol, consent, HIPAA authorization, IRB approval memos, etc.) as research involving prospective collection of data and/or biological specimens must be appropriately reviewed and approved by the local IRB and the ORP HRPO as a function of your contract/award.

None of the collection protocols referred to in response to the 2 questions above have or will receive funds from Proposal PC100802.

Additional information/documents will likely be required for the Carter submission too; for now, please submit the consent form under which the samples were procured.

Consent forms and IRB approval memos for the Partin and Trock protocols (Aim 1) and the consent form for the Carter protocol (Aim 2) are attached.

Has any of the prospective collection funded by this proposal/award been initiated?

No prospective collection is funded by this award. Only existing samples are used, and funds from this award were not used for the collection of those samples.

JHM IRB - eForm A – Protocol

1. Abstract

- a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

Explanatory Note: This application concerns use of existing human prostate biospecimens obtained from JHU researchers with IRB-approved protocols allowing storage and disbursement of biospecimens to other appropriate investigators for subsequent projects. These protocols are IRB # NA00047205 “Development and Evaluation of a Tumor Marker for Prostate Cancer”, PI: Alan Partin MD, PhD, and WIRB protocol #20011642 “Molecular Epidemiology of Prostate Cancer”, PI: Bruce Trock, PhD. Dr. Trock obtained tissues from these protocols for use in a Dept. of Defense funded study separate from the above 2 protocols (details of both protocols and DOD study below). Because Dr. Trock was a Co-Investigator on the grant that funded Dr. Partin’s protocol, and was actually the PI of the grant that funded the WIRB-approved biospecimen protocol he didn’t realize he would need separate IRB approval to use these biospecimens in the separate DOD-funded study. As part of the DOD-funded study he sent these biospecimens (without PHI) to a company that was contracted to perform biomarker analyses. **The purpose of this application is to correct this error** and ensure that the DOD-funded study has appropriate IRB approval for use of the biospecimens.

The DOD-funded study “Metabolomic Profiling of Prostate Cancer Progression During Active Surveillance” (to be referred to as “Metabolomics” for simplicity) was funded in 2011 for 3 years. Due to a number of complications it became necessary to apply to the DOD for a no cost extension (NCE) to finish the project. At the time the NCE was evaluated the lack of separate IRB approval for the Metabolomics study became apparent, prompting the current IRB application.

The goal of the Metabolomics study is to determine whether a metabolomic signature can be developed in either serum or urine that can augment prostate biopsy pathology to more accurately identify appropriate candidates for active surveillance (AS). AS is increasingly used for men with low grade (Gleason 6) prostate cancer whose tumors appear to exhibit an indolent phenotype unlikely to cause morbidity or death during their remaining natural lifespan. Appropriate management of prostate cancer by AS is an approach to reduce the harms of over-treatment. However, even though biopsy Gleason score is the predominant factor in determining whether a man is an appropriate candidate for AS (instead of immediate treatment), as a result of sampling error a biopsy indicating Gleason score 6 underestimates the true Gleason score (and hence the aggressiveness of the tumor) in anywhere from 20-40% of cases. **Our hypothesis** is that circulating metabolomic biomarkers can serve as another indicator of tumor phenotype that can augment biopsy information to identify men who may not be appropriate for AS despite a biopsy Gleason score = 6. Our industry contractor who will perform the actual metabolomics analyses on this project (Metabolon, Inc.) has identified a metabolomic profile in prostatectomy tissue that is associated with aggressive prostate cancer; the profile was derived from distinct patient cohorts independent of Johns Hopkins.

The aims of the Metabolomics study are: Aim 1: validate whether the signature from Metabolon, when measured in urine or serum, can distinguish samples that came from men with Gleason score 7 vs. Gleason score 6 at prostatectomy. Aim 2: use the signature in baseline samples of either serum or urine (whichever performs best in Aim 1) to determine whether it could distinguish men in AS who exhibited

biopsy upgrading to Gleason 7 or higher during follow-up (prompting a recommendation for treatment) compared to men whose biopsies continued to show only Gleason 6 during follow-up (remaining appropriate to continue AS).

2. Objectives (include all primary and secondary objectives)

Aim 1 will validate - in urine or serum – whether the metabolomic signature identified by Metabolon, Inc. can distinguish baseline samples that came from men with prostatectomy Gleason 6 vs. Gleason 7. This aim will use existing samples collected prior to surgery from men who undergo prostatectomy, under IRB protocol #NA00047205 “Development and Evaluation of a Tumor Marker for Prostate Cancer” (PI: Alan Partin, MD, PhD), and under WIRB protocol #20011642 “Molecular Epidemiology of Prostate Cancer”, PI: Bruce Trock, PhD.

Aim 2 will determine whether the metabolomic profile tested in Aim 1, when measured in baseline urine or serum samples from AS men, can distinguish those who do vs. do not undergo biopsy upgrading during follow-up (change during the course of annual repeat biopsies from Gleason 6 to Gleason 7; AS biopsy & follow-up protocol to be described in **Section 3** below). This aim will use existing samples from AS men collected under IRB protocol #NA00045103 “Active Surveillance for Prostate Cancer” (PI: H. Ballentine Carter, MD. Dr. Carter’s protocol allows samples to be stored for future research by qualified Johns Hopkins investigators.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Active Surveillance (AS) as an alternative to reduce over-treatment. Men are offered AS as an alternative to immediate treatment when their tumors appear to be very low risk, based on well-established biopsy parameters (see below). By carefully following men with a defined protocol of regular examinations and repeat (surveillance) biopsies, AS has as its goal to reduce over-treatment of tumors that may remain indolent over a man's lifetime, while not missing the window of opportunity for cure in men whose tumors appear to progress to a more aggressive phenotype. Although biopsy Gleason grade and tumor extent provide a good means of discriminating tumors with an indolent vs. aggressive phenotype, *our hypothesis is that molecular classifiers, specifically metabolomic profiles, can augment the biopsy pathology information* to significantly improve our ability to select men who are the most appropriate candidates who can safely be managed with AS.

Limitation of initial biopsy Gleason score to identify men with life threatening prostate cancer. It has long been known that in a significant fraction of cases, the biopsy Gleason score underestimates the true Gleason phenotype. In a study where men with biopsy Gleason 6 considering AS underwent repeat biopsy within ≤ 3 months 17% had Gleason score 7 (1). A second study of repeat biopsies at a median of 22 months after the initial biopsy of men in AS showed upgrading to Gleason score ≥ 7 in 35% (2). These results suggest that significant sampling error in the original diagnostic biopsy, rather than true histologic progression, is the major driver of upgrading to Gleason 7 observed in follow-up of AS patients (2). These data show that initial biopsy will misclassify a significant fraction of men who have higher risk disease that is not suitable for AS, and underscore the need to develop additional methods to characterize the tumor phenotype at enrollment into AS.

Active Surveillance (AS) Program at Johns Hopkins Hospital (JHH). The AS program at JHH is an established management option for men with very low risk prostate cancer; it is not considered

experimental or a clinical trial. At JHH AS is currently recommended for select individuals who meet the following criteria for very low risk (VLR): PSA density <0.15 ng/ml/cc, non-palpable prostate cancer (stage T1c), Gleason score of 6 or less with no grade 4 or above, no more than 2 cores positive for cancer, and no more than 50% of any one core involved with cancer (the initial biopsy must be at least 12 core and, if not performed at JHH, reviewed by JHH pathologists). Follow-up of men in the AS program involves a semiannual total and free PSA measurement, digital rectal exam, and an annual 12 core "surveillance" biopsy. "Biopsy reclassification" to where AS is no longer medically advisable and curative treatment is recommended occurs with any of the following adverse pathological features at surveillance biopsy: Gleason score ≥ 7 or any Gleason grade ≥ 4 (i.e. "biopsy upgrading"), or >2 cores positive for cancer, or $>50\%$ of any single core involved with cancer (i.e. increased tumor extent).

Metabolomic profiling of cancer. Metabolomics entails evaluation of the global metabolic profile; the patterns and concentration of metabolites over broad classes of compounds in a tissue or organ. These metabolites are small molecule products of biochemical reactions in a cell, represented by compounds in the mass range 80-1000 daltons. Although complementary to genomics, transcriptomics and proteomics, metabolomics may have advantages for defining phenotypes because it is downstream of changes in genes and proteins, and thus may be a better indicator of functional alterations in pathways affected by different pathological states. In this sense, metabolomic profiles represent the integration of genetic regulation, enzyme activity and metabolic reactions in a dynamic profile of the biological state of a tissue (3).

Our industry contractor, Metabolon, Inc. has developed a metabolomics signature from prostate tumor tissue that appears to differentiate aggressive from more indolent prostate cancer (4). Our study (**Aim 1**) will evaluate whether that signature can be defined in pre-surgical urine or serum samples and discriminate among those that came from patients with prostatectomy Gleason 7 vs. Gleason 6 tumors. We will then test the signature on serum or urine samples (whichever performed best in Aim 1) obtained from AS patients at entry to the AS program, to determine whether it can differentiate men who subsequently undergo biopsy upgrading compared to men whose repeat (surveillance) biopsies continue to show only Gleason 6 tumor.

4. Study Procedures

Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

For Aim 1 matched serum and urine samples were identified by Dr. Partin's team from among existing pre-surgical specimens contributed by men about to undergo prostatectomy, according to Dr. Partin's protocol #NA00047205. This protocol obtains serum and urine specimens to develop new tests for prostate cancer, and patients are informed that samples will be stored and used for future research. However, existing specimens from only 86 patients who met the requirements for the proposed study were identified (out of the 100 needed for aim 1; 50 Gleason 6 and 50 Gleason 7). Therefore, it was decided to obtain the remaining samples from existing samples collected under a separate protocol of Dr. Trock's for a previous Department of Defense-funded case-control study, "Molecular Epidemiology of Prostate Cancer" (award number DAMD17-00-1-0020), approved as WIRB protocol #20011642. This protocol was developed for a case-control study of diet and prostate cancer, but had been amended to allow blood and urine samples to be stored for future biomarker studies, and specifically mentioned metabolomics analyses as one possible type of study. By combining specimens from Dr. Partin's protocol #NA00047205 and Dr. Trock's protocol WIRB #20011642 the total of 100 specimens was achieved.

For Aim 1, samples from Dr. Partin were provided with an identification number that Dr. Trock cannot directly link to patients' Protected Health Information (PHI). The samples from Dr. Trock's protocol did

have identifiers, since Dr. Trock was the PI of the original study. Both serum and urine samples have volume 100-200 µl per patient. The samples have all been sent to Metabolon, Inc. for metabolomics analysis, using a study-specific ID number that can only be linked to patient data by Dr. Partin or Dr. Trock; **no PHI was sent to Metabolon, Inc.** The samples will be analyzed by Metabolon, Inc. and the metabolites in their previously defined signature (4) will be measured. Data will be sent to Dr. Trock for linkage to the clinical data and statistical analysis.

Aim 2 will use existing samples that were collected from men in the John Hopkins Active Surveillance program under IRB protocol #NA00045103 "Active Surveillance for Prostate Cancer" (PI: H. Ballentine Carter). Serum or urine samples (whichever performs better in Aim 1) in volumes of 100-200 µl per patient from 100 AS men who experienced biopsy upgrading during follow-up, and 100 men who continued on AS with all surveillance (follow-up) biopsies Gleason 6 or less will be identified from the stored samples. Samples from men who meet the study eligibility criteria have been identified by Dr. Carter's team, **but the samples will not be aliquotted or provide to Dr. Trock until IRB approval has been obtained**, and it is determined from Aim 1 whether the metabolomics signature performs better in serum or urine; information about these patients has not been provided to Dr. Trock at this time. After completion of Aim 1 the samples will be aliquotted and sent to Metabolon, Inc. for analysis, using study-specific ID numbers as in Aim 1. Metabolomic signature data will be sent to Dr. Trock for analysis.

a. Study duration and number of study visits required of research participants.

It is expected that the study will take one more year to complete metabolomics analyses and biostatistical data analyses. No visits or further contact are required of research participants, as all samples are existing samples previously donated.

b. Blinding, including justification for blinding or not blinding the trial, if applicable.

Personnel performing the metabolomics analyses at Metabolon, Inc. will be blinded to reduce the potential for bias. The data analysis to be performed by Dr. Trock will not be blinded.

c. Justification of why participants will not receive routine care or will have current therapy stopped.

Not applicable. Neither this study nor the studies under which the samples were originally collected affects patient care in any way.

d. Justification for inclusion of a placebo or non-treatment group.

Not applicable. This study does not involve treatment.

e. Definition of treatment failure or participant removal criteria.

Not applicable. This study does not involve treatment.

f. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Not applicable. This study does not involve treatment, and all patients have already contributed the samples to be used in the study.

5. Inclusion/Exclusion Criteria

Inclusion

- Men scheduled to undergo prostatectomy (Aim 1) or participating in Johns Hopkins AS program (Aim 2).
- Ability to give informed consent.

Exclusion

- Previous cancer other than non-melanoma skin cancer.
- Use of hormone modifying therapy within 6 months or less prior to donating blood and urine.

6. Drugs/ Substances/ Devices

- a. The rationale for choosing the drug and dose or for choosing the device to be used.

Not applicable. This study does not involve treatment.

- b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

Not applicable. This study does not involve treatment.

- c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

Not applicable. This study does not involve treatment.

7. Study Statistics

- a. Primary outcome variable.

Aim 1: Gleason 7 vs. Gleason 6 tumor.

Aim 2: Time to occurrence of biopsy upgrading (Gleason ≥ 7) vs. Gleason ≤ 6 maintained during follow-up biopsies.

- b. Secondary outcome variables.

None.

- c. Statistical plan including sample size justification and interim data analysis.

Metabolon, Inc. has identified a panel of 16 metabolites associated with aggressive phenotype in previous analyses in RP tissue. They will analyze these metabolites in the serum and urine samples and send the data to Dr. Trock.

Aim 1: Analyses will be the same for serum and urine samples. Univariate data analyses will compare each metabolite between Gleason 6 vs. 7 patients using Wilcoxon rank sum test. Metabolites with an association significant at $p < 0.20$ will be entered into a multivariable logistic regression model. Model discrimination will be determined by the area under the bootstrap-corrected ROC curve (AUROC). Comparison of the AUROC for serum vs. urine will determine which body fluid will be used in Aim 2 (5).

Aim 2: The metabolites used in the model with best performance in Aim 1 will be measured in samples from the AS patients. These will be entered into the model derived in Aim 1 to generate a risk score (predicted probability of Gleason 7). This risk score will be entered as a continuous variable into a Cox proportional hazards model for time to development of biopsy upgrading. Other known prognostic factors such as age, PSA density, number of positive biopsy cores will also be included in the model. The performance of the model will be determined by Harrell's concordance index, and a calibration curve will compare predicted vs. observed events (both bootstrap corrected for optimism (6)). The incremental improvement in model fit associated with the metabolomics signature will be determined by comparing to the c-index for the model that just includes the clinic-pathologic prognostic factors. We will also evaluate models that represent the metabolomics risk score as quartiles, or as a dichotomous variable determined by examining the ROC curve from Aim 1, and consultation with Dr. Carter about a cutpoint for predicted probability above which he would recommend against AS.

Power:

Power is based on the proportional hazards model, with the metabolomic profile used as a risk score dichotomized as Gleason ≥ 7 (high risk) vs. Gleason ≤ 6 (low risk). Power calculations are developed (table) for detecting minimally important hazard ratios (HR) based on 100 progressed and 100 non-progressed men. Because models will include potential confounding factors that may correlate with the metabolomic profile, we calculate power only for multivariable associations, where we conservatively assume a moderately strong correlation ($r=0.3$) between the metabolomic profile binary classifier and at least one other confounding variable (7). We calculate power for q_e = the prevalence of the "high risk" tumor status at 20% (quintiles), 30%, or 50% (dichotomized at the median) (8). All calculations assume $\alpha=0.05$, 2-sided test. It is important to note that we use HR=2.0 or 2.5 as a conservative basis for power calculation. We expect that the metabolomic classifiers will actually exhibit a stronger association with high grade, which would provide greater predictive ability to identify appropriate candidates for AS.

HR associated with high risk	Prevalence of high risk metabolomics signature (q_e)	Correlation with other prognostic factor (ρ)	Power
2.0	20%	0.3	0.753
2.0	30%	0.3	0.857
2.0	50%	0.3	0.911
2.5	20%	0.3	0.938
2.5	30%	0.3	0.980
2.5	50%	0.3	0.992

d. Early stopping rules.

Not applicable. Patients have donated samples at baseline and are not exposed to treatment or other study-associated risk.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

Not applicable. Patients have donated samples at baseline and are not exposed to treatment or other study-associated risk.

b. Steps taken to minimize the risks.

Risks associated with confidentiality are minimized by limiting access to PHI to appropriately authorized study Johns Hopkins investigators; no PHI is provided to Metabolon, Inc.

c. Plan for reporting unanticipated problems or study deviations.

Any unanticipated problems will be reported to the PI of the protocol under which the original specimens and data were collected, to the IRB, and to the DOD.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Consent forms under which samples and data were collected describe the measures to protect confidentiality and who is allowed to see the data, and state that complete confidentiality cannot be guaranteed. Given the protections that are in place, and that no further involvement of the study subjects beyond the initial specimen donation is required, the risk of breach of confidentiality is minimal.

e. Financial risks to the participants.

None. There are no costs to the participants.

9. Benefits

a. Description of the probable benefits for the participant and for society.

There are no benefits to the participants beyond altruism. Society may benefit if the samples and data contribute to identification of improved methods of classifying prostate cancer patients according to risk of aggressive disease.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Participants did not receive payment or remuneration for their original donation of specimens or consent to use their data, and there will be no payment or remuneration for those specimens and data to be used in the current protocol. There were no penalties

associated with not completing the protocol, or subsequently requesting that their specimens or data be removed.

11. Costs

- a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

There were no costs to participants for their original specimen donation or consent to use their data, and there will be no costs to participants for those specimens and data to be used in the current protocol.

References

1. Berglund RK, Masterson TA, Vora KC, et al. Pathological upgrading and up staging with immediate repeat biopsy in patients eligible for active surveillance. J Urol 2008; 180:1964-7.
2. Choo R, Danjoux C, Morton G, et al. How much does Gleason grade of follow-up biopsy differ from that of initial biopsy in untreated, Gleason score 4-7, clinically localized prostate cancer? Prostate 2007; 67:1614-20.
3. Spratlin JL, Serkova NJ, Eckhardt SG. Clinical applications of metabolomics in oncology: a review. Clin Cancer Res 2009; 15:431-440.
4. McDunn JE, Li Z, Adam KP, et al. Metabolomic signatures of aggressive prostate cancer. Prostate 2013; 73:1547-60.
5. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 1983; 148:839-43.
6. Harrell FE, Jr. Regression modeling strategies: with applications to linear models, logistic regression and survival analysis. Springer Verlag, NY, 2001.
7. Schmoor C, Sauerbrei W, Schumacher M. Sample size considerations for the evaluation of prognostic factors in survival analysis. Stat Med 2000; 19:441-452.
8. George SL, Desu MM. Planning the size and duration of a clinical trial studying the time to some critical event. J Chr Dis 1974; 27:15-24.



**Office of Human Subjects Research
Institutional Review Boards**

1620 McElderry Street, Reed Hall, Suite B-130
Baltimore, Maryland 21205-1911
410-955-3008
410-955-4367 Fax
e-mail: jhmirb@jhmi.edu

Date: July 15, 2016

APPLICATION APPROVAL

Review Type: Convened
Principal Investigator: Bruce Trock
Number: IRB00096293
Title: Metabolomic Profiling of Prostate Cancer Progression During Active Surveillance
Committee Chair: Howard Lederman
IRB Committee: IRB-1

Date of approval: July 12, 2016

Date of Expiration: July 11, 2017

The JHM IRB approved the above-referenced Application.

Approval includes an eForm A (dated 2/26/16), a consent waiver, a WIRB consent form and a data element document. The Board agrees that samples should not have been sent to Metabolon without having an IRB-approved protocol and would like to note that you should not have started to identify subjects for Aim 2 without an IRB-approved protocol. Please take note for future reference.

IRB review included the following:

45 CFR 46.116: A waiver of consent was granted based on the following criteria: 1) the research involves no more than minimal risk to subjects; 2) the waiver will not adversely affect the rights and welfare of the subjects; 3) the research could not be practicably carried out without the waiver; and 4) the IRB will advise you if it is appropriate for participants to be provided with additional pertinent information after participation.

Date of Approval and Expiration Date: The approval and expiration date for this research are listed above. If the approval lapses, the research must stop and you must submit a request to the IRB to determine whether it is in the best interests of individual participants to continue with protocol-related procedures.

Changes in Research: All proposed changes to the research must be submitted using a Change in Research application. The changes must be approved by the JHM IRB prior to implementation, with the following exception: changes made to eliminate apparent immediate hazards to participants may be made immediately, and promptly reported to the JHM IRB.

Continuing Review: Continuing Review Applications should be submitted at least 6 weeks prior to the study expiration date. Failure to allow sufficient time for review may result in a lapse of approval. If the Continuing Review Application is not submitted prior to the expiration date, your study will be terminated and a New Application must be submitted to reinitiate the research.

Unanticipated Problems: All unanticipated problems must be submitted using a Protocol Event Report.

If this research has a commercial sponsor, the research may not start until the sponsor and JHU have signed a contract.

Study documents:

Additional Supplemental Study Documents:


Data elements from Partin, Trock, and Carter protocols to be used in current study
Consent for WIRB20011642

Protocol:

eformA_v2_Trock_metabolomics2_26_16.doc

Study Team Members:

Alan Partin, Sacha Wolf, Patricia Landis, Leslie Mangold, H Carter

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT			1. CONTRACT ID CODE S		PAGE OF PAGES 1 13	
2. AMENDMENT/MODIFICATION NO. P00002		3. EFFECTIVE DATE 24-Aug-2016		4. REQUISITION/PURCHASE REQ. NO. W91ZSQ0317N636		5. PROJECT NO.(If applicable)
6. ISSUED BY USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014		CODE W81XWH		7. ADMINISTERED BY (If other than item 6) See Item 6		
8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code) JOHNS HOPKINS UNIVERSITY, THE 3400 N CHARLES ST W400 WYMAN PARK BLDG BALTIMORE MD 21218-2680				9A. AMENDMENT OF SOLICITATION NO.		
				9B. DATED (SEE ITEM 11)		
				X 10A. MOD. OF CONTRACT/ORDER NO. W81XWH-11-1-0451		
				X 10B. DATED (SEE ITEM 13) 30-Sep-2011		
CODE 5L406		FACILITY CODE				
11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS						
<input type="checkbox"/> The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer <input type="checkbox"/> is extended, <input type="checkbox"/> is not extended. Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.						
12. ACCOUNTING AND APPROPRIATION DATA (If required)						
13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS. IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.						
A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.						
B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).						
C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:						
X D. OTHER (Specify type of modification and authority) IAW USAMRAA General Terms and Conditions						
E. IMPORTANT: Contractor <input checked="" type="checkbox"/> is not, <input type="checkbox"/> is required to sign this document and return _____ copies to the issuing office.						
14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.) Modification Control Number: jmckean164636 PROJECT TITLE: Metabolomic Profiling of Prostate Cancer Progression During Active Surveillance PRINCIPAL INVESTIGATOR: Dr. Bruce Trock PERIOD OF PERFORMANCE: 30 September 2011 - 29 October 2017 AWARD AMOUNT: \$664,264 OBLIGATED AMOUNT: \$664,264 The purpose of this modification is to extend the period of performance until 29 October 2017. An annual technical progress report will be due on 29 October 2017. A final technical report will be due no later than 29 January 2018. Submission of SF425 financial reports shall continue on a quarterly basis throughout the extension period. All other terms and conditions remain unchanged.						
Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.						
15A. NAME AND TITLE OF SIGNER (Type or print)				16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print)		
				TERESA PARKER-REESER / GRANTS OFFICER		
				TEL: 301-619-2171 EMAIL: Teresa.M.ParkerReeser.civ@mail.mil		
15B. CONTRACTOR/OFFEROR		15C. DATE SIGNED		16B. UNITED STATES OF AMERICA		16C. DATE SIGNED
(Signature of person authorized to sign)				BY 		23-Aug-2016
				(Signature of Contracting Officer)		

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

The standard size code 1,000 has been added.
The NAICS code 541712 has been added.

CLIN 0001

The CLIN extended description has changed from:

Period of Performance: 30 September 2011-29 October 2014 (research ends on 29 September 2014)FY10 Prostate Cancer Research Program-Idea Development Award/Established Investigator

To:

Period of Performance: 30 September 2011-29 October 2017FY10 Prostate Cancer Research Program-Idea Development Award/Established Investigator.

DELIVERIES AND PERFORMANCE

The following Delivery Schedule item for CLIN 0001 has been changed from:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	DODAAC
POP 30-SEP-2011 TO 29-OCT-2014	N/A	USA MED RESEARCH MAT CMD 1077 PATCHEL STREET BLDG 1077 FORT DETRICK MD 21702 FOB: Destination	W91ZSQ

To:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	DODAAC
POP 30-SEP-2011 TO 29-OCT-2017	N/A	FORT DETRICK - CDMRP FORT DETRICK - CDMRP 1120 FORT DETRICK FREDERICK MD 21702 FOB: Destination	W91ZSQ

SECTION 00800 - SPECIAL CONTRACT REQUIREMENTS

The following have been modified:

Meeting abstracts during reporting period: None in connection with this project

Publications during reporting period: None in connection with this project

Manuscripts in preparation: None in connection with this project

Personnel receiving pay from this negotiated effort during Year 4:

Bruce Trock, PhD
Ballentine Carter, MD
Zhaoyong Feng, MS